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1 Cancer Prevention Group  
2 School of Cancer & Pharmaceutical Sciences  
3 Faculty of Life Sciences & Medicine  
4 King's College London

5 London, 19 June 2019

6  
7  
8 **16/18 GENOTYPING IN TRIAGE OF PERSISTENT**  
9 **HUMAN PAPILLOMAVIRUS INFECTIONS WITH NEGATIVE**  
10 **CYTOLOGY IN THE ENGLISH CERVICAL SCREENING PILOT**

11  
12 **Running title: HPV 16/18 triage for persistent infections**  
13

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51    **KEYWORDS**

52

53    Cervical cancer, screening, triage, high-risk human papillomavirus, genotyping

54

55

56 **ABSTRACT**

57

58 **Background.** In the English pilot of primary cervical screening with high-risk human  
59 papillomavirus (HR-HPV), we exploited natural viral clearance over 24 months to minimise  
60 unnecessary referral of HR-HPV+ women with negative cytology. Three laboratories were  
61 permitted to use 16/18 genotyping to select women for referral at 12-month recall. We estimated the  
62 clinical impact of this early genotyping referral.

63

64 **Methods.** The observed numbers of women referred to colposcopy and with detected high-grade  
65 cervical intraepithelial neoplasia (CIN2+), and of women who did not attend early recall in the three  
66 laboratories were compared with those estimated to represent a situation without an early  
67 genotyping referral. The 95% confidence intervals (CI) for the differences between the protocols  
68 were calculated by using a parametric bootstrap.

69

70 **Results.** Amongst 127,238 screened women, 16,097 (13%) had HR-HPV infections. The  
71 genotyping protocol required 5.9% (95% CI: 4.4-7.7) additional colposcopies and led to a detection  
72 of 1.2% additional CIN2+ (95% CI: 0.6-2.0), while 2.3% (95% CI: 2.1-2.5) fewer HR-  
73 HPV+/cytology- women did not attend the early recall compared with the non-genotyping  
74 protocol.

75

76 **Conclusions.** In a screening programme with high quality of triage cytology and high adherence to  
77 early recall, 16/18 genotyping of persistent HPV infections does not substantially increase CIN2+  
78 detection.

79

80

## 81 BACKGROUND

82

83 In England, the National Health Service (NHS) has provided cervical screening since 1988 through  
84 a “call and recall” Cervical Screening Programme (CSP). Women become eligible for screening at  
85 age 25 years. Thereafter, they are recalled for cytological screening every three years until age 50  
86 years, and then five yearly until the age of 64 years. Nationwide roll-out of primary high-risk  
87 human papillomavirus (HR-HPV) screening triaged with cytology is planned to be implemented by  
88 the end of 2019. In 2013, a pilot of primary cervical screening with HR-HPV testing was set up in  
89 six large CSP laboratories, accounting for about 13% of the nationally screened population.<sup>1</sup>

90

91 The aim of substituting cytology with HR-HPV testing is to achieve greater sensitivity and increase  
92 screening intervals. Because of relatively poor specificity, however, reflex cytology is required to  
93 identify those HR-HPV+ women who require colposcopy referral. In several countries including the  
94 USA and Australia, HPV 16/18 genotyping is being used at baseline to identify women with  
95 negative cytology at increased risk of underlying cervical intraepithelial neoplasia (CIN), for  
96 immediate referral.<sup>2, 3</sup> In these cases, the decision to refer HPV 16/18 positive women is made on a  
97 single screening sample.

98

99 The English pilot also recognised a potential value of HR-HPV genotyping in triage, but it was  
100 considered that implementing it in the same way as those other countries, i.e. based on a single  
101 sample, would lead to an unsustainable increase in the demand for colposcopy. Hence, women with  
102 HR-HPV infections have been managed as shown in Table 1. At baseline and at 12-month early  
103 recall, the selection of HR-HPV positive women for colposcopy relied on positive cytology, defined  
104 as borderline change in squamous or endocervical cells or worse. This is equivalent to atypical  
105 squamous cells of undetermined significance (ASCUS, and atypical glandular cells of undetermined  
106 significance, AGUS, in the Bethesda 2014 classification), or worse. Evidence of 24-month

107 persistence of a HR-HPV infection, regardless of concurrent cytology, also triggered referral for  
108 colposcopy. Additionally, three of the six laboratories used HPV 16/18 genotyping as a basis for  
109 more rapid referral for colposcopy in cases where there was persistent infection at the 12-month  
110 early recall in the absence of cytological abnormality. This means that a decision to refer cytology-  
111 negative women to colposcopy based on 16/18 genotyping is made only after two consecutive HR-  
112 HPV positive samples.

113

114 Both of these triage protocols were aimed at reducing the need for colposcopy by exploiting the  
115 substantial natural clearance rates of all HR-HPV infections, including HPV 16/18.<sup>4-6</sup> The non-  
116 genotyping protocol with two early recalls within 24 months after screening, aimed to maximise the  
117 reduction in the need for colposcopy but was potentially vulnerable to the risk of non-adherence  
118 with an additional early recall. The genotyping protocol, expediting a referral of women with the  
119 most high-risk infections and reserving the second early recall for those whose infections are less  
120 likely to progress to cancer, aimed to reduce loss to follow-up at the second early recall and to  
121 maximise the detection of CIN2+ lesions requiring treatment. Hence, we evaluated the differences  
122 between the two protocols in the overall frequency of referral for colposcopy, detection of CIN2+  
123 and CIN3+, and the loss to follow-up at early recall.

124

## 125 **MATERIALS AND METHODS**

126

127 The pilot

128

129 The pilot started in May 2013 and the main outcomes have been described previously in detail.<sup>7</sup>  
130 Briefly, six English CSP laboratories converted around a third of their screening population from  
131 primary liquid-based cytology (LBC) to primary HR-HPV screening. Conversion was population-  
132 based. The selection of administrative areas for conversion was not determined in a random process.

133 Rather, the laboratories considered practical issues such as maintaining a single clinical  
134 management protocol in colposcopy practices serving each administrative area. During the pilot, the  
135 population age range and recommended screening intervals remained unchanged.

136

137 Screening and diagnostic tests

138

139 Screening samples were taken within primary care and were collected in either SurePath (Becton  
140 Dickinson, Sparks, MD) or ThinPrep (Hologic, Marlborough, MA) LBC media. SurePath was used  
141 in three laboratories, while ThinPrep was used in the other three. In 2013-2014, two laboratories  
142 used Cobas 4800 (Roche, Rotkreuz, Switzerland, or Branchburg, NJ); two used RealTime (Abbott,  
143 Wiesbaden, Germany); and the remaining two used APTIMA (Hologic, Manchester, UK). Cobas  
144 and RealTime are HR-HPV DNA genotyping assays that report HPV 16 and HPV 18 separately  
145 from the 12 other HR-HPV genotypes, which are reported in combination. APTIMA is an HR-HPV  
146 mRNA assay detecting the 14 HR-HPV genotypes in combination.

147

148 All HR-HPV assays had previously been approved for primary screening within the CSP. Triage  
149 cytology was read under routine conditions with knowledge of a HR-HPV infection, and was  
150 quality controlled to CSP standards. Colposcopy was conducted according to national clinical  
151 practice guidelines. All diagnoses reflect routine cytopathology and histopathology in the CSP.

152

153 Study design

154

155 The present study was designed to compare the outcomes of screening in the pilot with and without  
156 HPV 16/18 triage at the 12-month early recall. As the first screening invitation is sent at age 24.5  
157 years, we included women aged 24-64 years at the time of the screening test. Additionally, women  
158 were included if they had been screened during the first (prevalence) round of primary screening



159 with HR-HPV testing from the beginning of the pilot in May 2013 until December 2014 in the three  
160 Cobas or RealTime laboratories that used the HR-HPV genotyping information for the management  
161 of HR-HPV positive women (Table 1). Data on all subsequent tests and diagnoses were retrieved  
162 from the laboratories' information systems until May 2017, which gave all women 29-49 months of  
163 follow-up after the primary screening test.

164  
165 Women screened in the three laboratories that did not use HR-HPV genotyping information for the  
166 management of HR-HPV positive women were not included as a comparator in this post-hoc  
167 analysis. Two of these laboratories used the APTIMA assay. Unlike DNA assays that typically  
168 detect both transient infections and those integrated into a host's genome, APTIMA has been  
169 designed to detect (predominantly) the latter type of infections. It has indeed been observed that this  
170 assay typically detects fewer HR-HPV infections than DNA assays, which ultimately leads to lower  
171 colposcopy rates in a routine screening programme.<sup>8,9</sup> Consequently, using APTIMA data as a  
172 comparator would have introduced the effect of the assay's different molecular target into the  
173 comparison of the triage protocols and hence could substantially affect analysis, particularly in  
174 terms of the number of colposcopies.

175  
176 The prevalence screening episode for each woman was defined as starting with the first test  
177 recorded during the pilot period, i.e. the primary (baseline) test, and closed with any early recall  
178 tests or colposcopies. If the first recorded pilot test was preceded by another test within the two  
179 prior years, or if the test's management code identified it as a follow-up to a recent cervical  
180 abnormality, the episode was excluded from further analysis. This is because those tests were  
181 unlikely to have been taken for the purpose of primary screening. Tests were linked using each  
182 women's unique English NHS numbers.

183

184 In this analysis, the infecting HR-HPV genotype was determined at the primary test and remained  
185 fixed even if the genotype changed by the 12-month early recall. The effect of a genotype change  
186 on the studied outcomes was addressed in a sensitivity analysis (see below). Women were included  
187 in the 16/18 category regardless of any co-infecting genotypes.

188  
189 Our primary endpoints were (1) the total number of colposcopies performed, (2) the number of HR-  
190 HPV positive/cytology negative women not adhering to early recall, and (3) the number of detected  
191 CIN2+ lesions for each triage protocol. CIN2+ was chosen as one of the primary endpoints as this is  
192 the threshold for treatment, but the results are also presented for the more reproducible endpoint of  
193 CIN3+. <sup>10</sup>

194  
195 These outcomes were estimated based on aggregated observed data from the three genotyping  
196 laboratories (Table 2), and the following two sets of assumptions. Firstly, we assumed that all  
197 women would be referred as expected on the basis of their screening outcomes (Table 1). For a  
198 minority of women in the data where this did not happen (gray cells in Table 2), we assumed that  
199 they would have the same clinical outcomes as women who were referred as expected. As this was  
200 done consistently for both protocols, the calculated total numbers of colposcopies, CIN, and women  
201 not returning for early recall under the genotyping protocol differ slightly from those that were  
202 directly observed. Secondly, the 24-month outcomes in cytology-negative women persistently  
203 infected with HPV 16/18 at 12 months could not be directly observed for the non-genotyping  
204 protocol. We estimated them on the following assumptions: a) that attendance at 24-month early  
205 recall and colposcopy would be the same as that observed among women infected with other HR-  
206 HPV genotypes, b) that persistence of HR-HPV infections between the 12- and 24-month early  
207 recalls would be as that observed in a fourth pilot laboratory, which reported HR-HPV genotyping  
208 data but implemented a non-genotyping triage protocol (Table 1), and c) that CIN2+ and CIN3+

209 prevalent at 12-month early recall would still be detectable at 24-month early recall, i.e. that there  
210 was no excess regression or progression between the two early recalls.<sup>11</sup>

211

212 Two sensitivity analyses were undertaken to assess the robustness of the findings. In the first of  
213 these, we addressed a subgroup of women with HPV 16/18 infections and persistently negative  
214 cytology at 12 months. Among these women, a relatively large proportion did not have a record of  
215 referral for colposcopy (Table 2). In the base case analysis, we assumed that this was at random. In  
216 the sensitivity analysis, we used two conventional extreme assumptions for parameters with  
217 uncertain true values, i.e. that (analysis S1a) all women in this subgroup would have attended  
218 colposcopy with CIN2+ detection doubled from the (observed) base case value; or (analysis S1b)  
219 only half of the women in this subgroup would attend with CIN2+ detection halved from the base  
220 case value. A lower CIN2+ detection could be expected, for example, in cases where HPV 16/18  
221 infection had cleared by the 12-month early recall, but the woman remains HR-HPV positive.  
222 Indeed, this situation represented about two-thirds of the women without a record of referral to  
223 colposcopy at 12 months in the observed data. In the second sensitivity analysis, persistence of  
224 infections between the 12- and 24-month early recalls in women with negative cytology and HPV  
225 16/18 infections (which played a role in estimating the number of colposcopies in the non-  
226 genotyping protocol) was based on a small dataset from a single laboratory (N=98). We varied the  
227 proportion of women with persistent infections as: (analysis S2a) the lower 5% confidence limit; or  
228 (analysis S2b) the upper 95% confidence limit.

229

230 Statistical analysis methods

231

232 For both the number of colposcopies and the number of CIN2+ lesions detected, the relative  
233 difference was reported as the ratio between the absolute difference in the totals for the genotyping  
234 and the non-genotyping protocols (numerator) and the total number in the non-genotyping protocol

(denominator). For the number of women not adhering to early recall, the total number with HR-HPV positive cytology negative samples at baseline was used as the denominator. The positive predictive value (PPV) of colposcopy for CIN2+ and CIN3+ was calculated using the number of women attending colposcopy as the denominator. Detailed formulae are reported in Supplementary information.

We obtained 95% confidence intervals (CI) for detection of CIN2+, number of colposcopies and loss of adherence to follow-up at the 12- and 24-month early recall using a parametric bootstrap. More precisely, following the flows in Figure 1, we sampled the numbers in each category based on the observed data in Table 2; this process was repeated 10,000 times and the empirical distributions of the resulting numbers of colposcopies, CIN2+ and CIN3+, and women not attending early recall were used to form a 95% CI. The statistical software R (version 3.4.1) was used for all analysis.<sup>12</sup>

**RESULTS**

**Observed screening outcomes by HR-HPV genotype**

In total, 127,238 women were screened in the three genotyping laboratories in 2013-2014. Of these, 16,097 (13%) had a positive HR-HPV test result, 5287 (4%) with positive and 10,810 (8%) with negative cytology (Table 2). In total, 8759 (7%) HR-HPV positive women underwent a colposcopy, leading to detection of 2859 (2%) CIN2+ and 1763 (1%) CIN3+ (Table 3 and Figure 1). These numbers include detection following the recommended management protocol, including early recall as well as any colposcopies undertaken outside the protocol. Colposcopies and CIN observed outside of the recommended protocol, for example those after an immediate referral of HR-HPV positive cytology negative women at baseline, were infrequent and were not included in further

analyses. They amounted to 310 (4%) colposcopies, 31 (1%) CIN2+, and 16 (1%) CIN3+ (Figure 1).

Detection of CIN2+ was highest amongst women screened at age 24-29 years: 6.6% and 4.1% for CIN2+ and CIN3+, respectively. By comparison, the numbers were 1.6% and 1.0% at 30-49, and 0.5% and 0.3% at 50-64 years of age. A case of CIN2+ was detected for every three colposcopies at age 24-29 years and for every six colposcopies at age 50-64 years. For CIN3+, the numbers of colposcopies needed at these ages were four and 10 per case, respectively (data not tabulated).

Half of all CIN2+ (50%, 1423/2859) and 55% (968/1763) of CIN3+ were diagnosed in women infected with HPV 16, whereas 9% (247/2859 and 153/1763) of CIN2+ and CIN3+ were detected in women with HPV 18 without HPV 16 (Table 3). Other genotypes without either HPV 16 or 18 were detected in 41% (1189/2859) of CIN2+ and 36% (642/1763) of CIN3+. Amongst all 4047 women infected with HPV 16, 35% (1423/4047) were ultimately diagnosed with CIN2+ and 24% (968/4047) with CIN3+. For the 1160 women infected with HPV 18, this was 21% (247/1160) and 13% (153/1160), respectively, and for the remaining 10,890 women with other HR-HPV infections it was 11% (1189/10,890) and 6% (642/10,890), respectively (Table 3).

During the same period, the fourth laboratory with HR-HPV DNA genotyping information, but implementing a non-genotyping triage protocol, screened 15,831 women with HR-HPV testing. Of these, 1714 (11%) had a positive HR-HPV test result, 1274 (8%) with negative and 440 (3%) with positive cytology. This was similar to the screening results in the three substantially larger laboratories included in the main analysis. Among the 98 women with HPV 16/18 infections and negative cytology persisting at 12 months, the infection persisted until 24 months in 73 (74%). This proportion was virtually constant across age groups (data not tabulated). Among women with HPV 16/18 infections who attended colposcopy after the 24-month early recall, the observed PPV for

286 CIN2+ was 27% (19/71), with 15% (8/54) if they had negative cytology, and 65% (11/17) if they  
287 had positive cytology.

288

#### 289 **Estimating the impact of the genotyping triage protocol**

290

291 The genotyping protocol generated detection of 2869 CIN2+ and 1769 CIN3+ resulting from 8750  
292 colposcopies among the 127,238 screened women (Table 4). More than 90% of all CIN2+ (91%,  
293 2614/2869) were detected after a referral with positive cytology at either the baseline test or at the  
294 12-month early recall. An additional 5% (133/2869) of CIN2+ were detected after a referral of HPV  
295 16/18 positive women with persistently negative cytology at 12 months, and the final 4%  
296 (123/2869) of CIN2+ were diagnosed at 24-month early recall amongst women persistently infected  
297 with other HR-HPV genotypes. This pattern was very similar for the detection of CIN3+.

298

299 An estimated 1741 cytology negative women with a positive baseline HR-HPV test result did not  
300 attend the 12-month early recall. Additionally, 637 women who attended the 12-month early recall  
301 did not attend a recommended 24-month early recall. In total, we estimate that 22% (2378/10,810)  
302 of HR-HPV positive cytology negative women did not attend or complete early recall.

303

#### 304 **Estimating the impact of the non-genotyping triage protocol**

305

306 With this protocol, a total of 2835 CIN2+ and 1751 CIN3+ would be detected as a result of 8260  
307 colposcopies among the 127,238 screened women (Table 4). Again, >90% of all high-grade CIN  
308 would be detected following positive triage cytology at baseline or at 12-month early recall. The  
309 remaining CIN2+ would be detected at the 24-month early recall for persistent HR-HPV.

310

311 Referring all persistently HR-HPV positive women with negative cytology at 12 months to an  
312 additional 24-month early recall would result in 8% (864/10,810) of women not attending, in  
313 addition to the 16% (1741/10,810) not attending the 12-month early recall. In total, we estimate that  
314 24% (2626/10,810) of HR-HPV positive cytology negative women would not have completed the  
315 recall under the non-genotyping triage protocol.

316

#### 317 **PPV of a referral for colposcopy**

318

319 The PPVs for CIN2+ were high when a colposcopy was undertaken following a positive cytology  
320 triage test result: 41% (2135/5163) at baseline and 35% (479/1369) after the 12-month early recall  
321 (Table 4).

322

323 In women infected with non-16/18 HR-HPV genotypes referred after the 24-month early recall, the  
324 PPV of a colposcopy was 10% (123/1198; Table 4). At this point, positive cytology was not used as  
325 a condition for a colposcopy. Nevertheless, the laboratories did report the cytology grade and the  
326 PPV for CIN2+ remained high, 29% (66/228), amongst women with cytological abnormalities, and  
327 much lower, 6% (51/907), amongst women who remained cytologically negative (data not  
328 tabulated; cytology of the remaining 9 out of 1144 women with a colposcopy (Table 2) was not  
329 graded).

330

331 In women with HPV 16/18 positive persistently negative cytology, the PPV for CIN2+ was 13%  
332 (133/1020) at the 12-month early recall. At 24 months, the PPV for persistent HPV 16/18  
333 infections, regardless of cytology, is estimated at 18% ((221-123)/(1728-1198), Table 4). The PPV  
334 could not be reliably estimated separately by cytology but as reported earlier, it was 15% among 54  
335 cytology negative women in the fourth genotyping laboratory.

336

337 In all cases, the PPVs for CIN3+ were approximately half those for CIN2+.

338

### 339 **Comparison of the two protocols**

340

341 We estimate that the genotyping protocol would detect an additional 34 (95% CI: 26-43) CIN2+  
342 and 18 (95% CI: 13-24) CIN3+ cases among the 127,238 screened women, representing 1.2% (95%  
343 CI: 0.9-1.5) of CIN2+ and 1.0% (95% CI: 0.8-1.4) of CIN3+ cases detectable by the non-  
344 genotyping protocol (Table 5). It would result in 5.9% (95% CI: 5.0-6.9) more colposcopies; 8750  
345 (95% CI: 8572-8924) vs. 8260 (95% CI: 8079-8444), a difference of 490 (95% CI: 420-562). It  
346 would also result in 2.3% (95% CI: 2.1 to 2.5) fewer HR-HPV positive cytology normal women not  
347 completing their recommended early recall; 2378 (95% CI: 2283-2475) vs. 2626 (95% CI: 2520-  
348 2731), a difference of 248 (95% CI: 226-270). The differences between the two protocols were very  
349 similar across all age groups (Table 5).

350

351 The outcomes were not materially affected by varying the assumptions on the attendance at  
352 colposcopy and prevalence of CIN2+ in HPV 16/18 positive women with persistently negative  
353 cytology. Under the favourable scenario for the genotyping protocol (analysis S1a: a high  
354 attendance at colposcopy and a high PPV), the latter would increase the need for colposcopy by  
355 6.1% (95% CI: 5.2-7.0) and CIN2+ detection by 1.6% (95% CI: 1.3-1.9). Under the unfavourable  
356 scenario (analysis S1b: a low attendance at colposcopy and a low PPV), the estimates would be  
357 lower at 4.7% (95% CI: 3.8-5.6) and 0.3% (95% CI: 0.1-0.6), respectively. Varying the proportion  
358 of women infected with HPV 16/18 who remain HR-HPV positive by 24 months produced a range  
359 in the extra demand for colposcopy between 6.6% (analysis S2a, 95% CI: 5.6-7.6) and 5.3%  
360 (analysis S2b, 95% CI: 4.5-6.1).

361

362



## 363 **DISCUSSION**

364

365 Using data from the English HPV pilot we estimated there would be a small increase in CIN2+  
366 detection for HPV 16/18 genotyping compared with non-genotyping triage protocols for women  
367 with persistent HR-HPV infections and negative cytology. However, more rapid referral of  
368 persistently HPV 16/18 positive women with negative cytology would increase the number of  
369 colposcopies by 6%, which appears to be disproportionate with respect to an estimated increase in  
370 detected CIN2+ of 1%. This is a consequence of both reasonably high compliance with repeated  
371 testing in early recall observed in the pilot (close to 80%), and highly sensitive stratification of risk  
372 by cytology triage. The latter identified 75% of all CIN2+ at baseline and an additional 17% at 12-  
373 month early recall, with a high PPV on both occasions of over 30%. A very small pool of CIN2+  
374 remained to be identified solely by HR-HPV genotyping but the PPV was substantially lower at  
375 around 10%.

376

377 As HPV 16/18 lesions are more likely to progress to cancer,<sup>13-15</sup> our finding of a 1% higher  
378 detection of CIN2+ and CIN3+ with a faster referral of HPV 16/18 positive women warrants  
379 consideration. This relatively small additional increase in the number of detected CIN2+ achieved  
380 by genotyping persistent HR-HPV infections would be observed on top of the approximately 50%  
381 increase achieved in the pilot by substituting cytology with HR-HPV testing,<sup>7</sup> and most of these  
382 cases would be detected in women below 30 years of age, when the likelihood of regression of  
383 CIN2+ is highest.<sup>16</sup> Persistently negative cytology is often associated with early infections and  
384 lesions detectable only through HR-HPV testing have been hypothesised to be small.<sup>17</sup> Given the  
385 long duration of progression of CIN lesions to cervical cancer,<sup>16, 18, 19</sup> a delay of 12 months in  
386 diagnosing these cytologically negative lesions is unlikely to be associated with a significant risk of  
387 interval cancer, provided women adhere to early recall.

388

389 HPV 16/18 genotyping has been recommended for an immediate referral of HR-HPV  
390 positive/cytologically negative women in countries such as the USA<sup>2, 20</sup> and Australia.<sup>3</sup> In Europe,  
391 the attitude towards using genotyping in this manner has so far been more conservative,<sup>21-23</sup> and  
392 baseline referral was not tested in the English pilot out of concern that it would lead to an  
393 unsustainable demand for colposcopy. When the switch was made from cytology to HR-HPV  
394 screening in the pilot, the demand for colposcopy increased by about 80% in the prevalence round.<sup>7</sup>  
395 Had direct referral of all HPV 16/18 positive women been recommended, we estimate that referral  
396 would increase by an additional 15-20% (Supplementary information). As expected, viral clearance  
397 however was substantial (32% of women with HPV 16/18 infections and negative cytology tested  
398 HR-HPV negative at the 12-month early recall, and a further 26% tested negative at the 24-month  
399 recall). The immediate colposcopies in women destined to clear their infections are likely to have  
400 contributed to the very high average number of colposcopies needed to detect each CIN2+ case in  
401 the ATHENA study, which evaluated a setting with immediate colposcopy of all women aged  $\geq 25$   
402 years with HPV 16/18 infections; this number was eight.<sup>11</sup> In the English pilot, where cytologically  
403 negative women were only referred in the presence of a persistent infection, the number of  
404 colposcopies to detect a case of CIN2+ was three (8750/2869, Table 4).

405

406 Birth cohorts vaccinated against HPV 16/18 in the catch-up programme did not start entering the  
407 CSP until 2015, which means that our analysis is representative of an unvaccinated population.  
408 Through cross-protection, vaccination has the potential to decrease not only the prevalence of HPV  
409 16/18 but also of certain other HR-HPV genotypes.<sup>24</sup> As a result, the overall number of screened  
410 women who will require triage and colposcopy will decrease. The value of using genotyping for  
411 HPV 16/18 in the remaining persistent infections will probably decrease in line with the expected  
412 decrease in CIN2+ lesions associated with HPV 16/18.<sup>25</sup>

413

414 The large size and prospective protocol are key strengths of our study, as well as a population-  
415 based, routine HR-HPV based screening setting using national standards and clinical guidelines,  
416 with quality assured HR-HPV testing, cytology, colposcopy, and histology. The patterns of  
417 detection of CIN2+ by genotype (Table 3) were consistent with the literature. We were limited by  
418 having access to data from the laboratories participating in the pilot; if women moved away from  
419 the catchment areas of these six laboratories, their subsequent outcomes could not be traced.  
420 Nevertheless, the completeness of follow-up was high, about 95% after a referral for a colposcopy  
421 and about 80% after a referral for an early recall (Figure 1). We could not directly observe the  
422 outcomes of a non-genotyping protocol. The resulting post hoc nature of our analysis required us to  
423 make several, albeit standard,<sup>11</sup> assumptions on infection dynamics and the prevalence of CIN in  
424 women when managed following the non-genotyping protocol. Nonetheless, the sensitivity analyses  
425 showed that our conclusions were robust against a variety of assumptions. Additionally, using the  
426 data from the same three laboratories for both triage protocols meant that the background  
427 characteristics of the women, the catchment areas' screening coverage, and the cytology reading  
428 practices were constant. Finally, while our study compared two defined triage protocols, it cannot  
429 provide a conclusive answer as to what the optimal triage strategy would be for English HR-HPV  
430 positive women. A full optimisation study would require a substantially different approach  
431 comparing a number of alternative strategies, varying e.g. the eligibility criteria for triage, the  
432 number of early recalls, their timing, the tests and their positivity thresholds, and any age  
433 stratification.<sup>26</sup> This is beyond the scope of our analysis.

434

## 435 **CONCLUSION**

436

437 In population based screening programmes with good quality of triage cytology and where most  
438 women adhere to early recall, HPV 16/18 triage of persistently HR-HPV positive and cytologically

439 negative women 12 months after primary screening can add very little in terms of a clinical benefit  
440 such as additional detection of CIN2+.  
441  
442

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447    Giles, Katherine Hunt, Sue Moss (past member), Janet Parker, Julietta Patnick (past member), Janet  
448    Rimmer, David Smith, Ruth Stubbs, and Penelope Tidbury.

449

450    **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

451    Women participating in the HPV primary screening pilot were invited to make an informed choice  
452    on participating in the cervical screening programme. A decision is made to accept or decline a  
453    screening test based on access to accurate and up-to-date information on the condition being  
454    screened for, the testing process and potential outcomes. Specific information was provided at the  
455    invitation stage allowing for personalised informed choice. There was further opportunity to reflect  
456    on what the test and its results might mean when they attended for screening with the clinician  
457    taking the sample. Regulation 5, Health Service Regulations 2002, Confidentiality Advisory Group  
458    Reference: 15/CAG/0207, was the legal basis to process the data.

459

460    **CONSENT FOR PUBLICATION**

461    Not applicable.

462

463    **DATA AVAILABILITY**

464    No additional unpublished data are available from the authors. Requests for access to data should be  
465    made to Public Health England, Office for Data Release.

466

467

468

469

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471 The funding for the pilot and the epidemiological evaluation thereof was provided by PHE. PHE  
472 had a role in designing the pilot; in the collection of the data; and commented on the manuscript.  
473 MR and HK made the final decision to submit.

474

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476 ARB was funded through Cancer Research UK, grant number C569/A16891.

477

478 **CONFLICT OF INTEREST**

479 MR: PHE provided financing for the epidemiological evaluation; attended meetings with various  
480 HPV assay manufacturers; fee for lecture from Hologic paid to employer.

481

482 ARB: No conflict of interest to disclose.

483

484 CM: Partly funded by PHE that provided financing for the epidemiological evaluation.

485

486 KD has received speaker fees and travel expenses to attend meetings from Hologic.

487

488 MH, TL, XT: PHE provided funding to support the NHS screening laboratory activity for the pilot.

489

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491 accommodation from Roche for training and from Abbott for user group meeting; Roche, Abbott,  
492 Hologic, Becton Dickinson and Cepheid provided kits for assay validation purposes; PHE provided  
493 funding to support the NHS screening laboratory activity for the pilot.

494

495 JS: Personal speaker bureau fees from Beckton Dickinson; personal medical advisory board fees  
496 from Zilico.

497

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500 impedance spectroscopy in detection of cervical intraepithelial neoplasia with Zilico.

501

502 HK: Chair of the Advisory Committee for Cervical Screening (PHE), but the views expressed in  
503 this manuscript are those of the author and do not represent the view of PHE.

504

#### 505 **AUTHORS' CONTRIBUTIONS**

506 Study design: MR, HK

507 Data management: CM

508 Statistical analysis: MR, AB

509 Drafted the paper: MR, HK

510 Commented on the draft: all authors

511 Decision to submit: all authors

512

513

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601

602 Table 1. Management of women in the English pilot of primary cervical screening with HR-HPV  
 603 testing.

Time of testing	Genotyping triage	Non-genotyping triage <sup>b</sup>
Baseline test	HR-HPV negative: routine recall at 3/5 years <sup>a</sup> HR-HPV positive/positive cytology: colposcopy HR-HPV positive/negative cytology: early recall at 12 months	
Early recall at 12 months	HR-HPV negative: routine recall at 3/5 years <sup>a</sup> HR-HPV positive/cytology positive: colposcopy	
	HPV 16/18 positive/cytology negative: colposcopy Other HR-HPV positive/cytology negative: early recall at 24 months	HR-HPV positive/cytology negative: early recall at 24 months
Early recall at 24 months	HR-HPV negative: routine recall at 3/5 years <sup>a</sup> HR-HPV positive: colposcopy	

604 <sup>a</sup> Depending on the woman’s age. The three-year routine recall interval is used for women aged 25-49 years, whereas  
 605 the five-year interval is used for women aged 50-64 years.

606 <sup>b</sup> One of the laboratories recorded HR-HPV genotyping information using a DNA assay but did not use it for clinical  
 607 management of HR-HPV positive women.

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613 Table 2. Observed outcomes for HR-HPV positive women in the three genotyping laboratories combined.

	N	Yes	Unknown	Proportion Yes
<b>Baseline</b>				
HR-HPV+	127,238	16,097	258	12.7%
<i>Cytology+ if HR-HPV+</i>	16,097	5287	0	32.8%
Had colposcopy if HR-HPV+/cytology+ after a record of referral	5287	5163	0	97.7%
PPV of colposcopy for CIN2+ if HR-HPV+/cytology+	5163	2135	0	41.4%
PPV of colposcopy for CIN3+ if HR-HPV+/cytology+	5163	1367	0	26.5%
<b>Early recall at 12 months (HR-HPV+/cytology- at baseline)</b>				
Had early recall testing after a record of referral	10,810	8964	125	83.9%
HR-HPV+	8964	5263	0	58.7%
<i>Cytology+ if HR-HPV+</i>	5263	1410	23	26.8%
Had colposcopy if HR-HPV+/cytology+ after a record of referral	1410	1353	0	96.0%
PPV of colposcopy for CIN2+ if HR-HPV+/cytology+	1353	473	0	35.0%
PPV of colposcopy for CIN3+ if HR-HPV+/cytology+	1353	269	0	19.9%
<i>Cytology- if HR-HPV+</i>	5263	3830	23	72.8%
HPV 16 or 18+ if HR-HPV+/cytology-	3830	1072	0	28.0%
Had colposcopy if HPV 16 or 18+/cytology- after a record of referral	1072	789	233	94.0%
PPV of colposcopy for CIN2+ if HPV 16 or 18+/cytology-	789	103	0	13.1%
PPV of colposcopy for CIN3+ if HPV 16 or 18+/cytology-	789	55	0	7.0%
<b>Early recall at 24 months (HR-HPV other+/cytology- at baseline and HR-HPV+/cytology- at 12-month early recall)</b>				
Had early recall testing after a record of referral	2758	2091	48	77.2%
HR-HPV+	2091	1368	0	65.4%
Had colposcopy after a record of referral	1368	1144	23	85.1%
PPV of colposcopy for CIN2+ if HR-HPV+	1144	117	0	10.2%
PPV of colposcopy for CIN3+ if HR-HPV+	1144	56	0	4.9%
<b>Early recall at 24 months (HPV 16 or 18+/cytology- at baseline and HR-HPV+/cytology- at 12-month early recall)<sup>a</sup></b>				
HR-HPV+	98	73	0	74.5%

614 CIN: cervical intraepithelial neoplasia. HR-HPV: high risk human papillomavirus; any of the 14 high risk genotypes detectable by the Cobas and RealTime assays unless otherwise

615 specified. PPV: positive predictive value.

616 Gray cells: Proportions of women who adhered to the type of clinical follow-up recommended by the protocol, calculated after exclusion of category “unknown” from the  
617 denominator (if non-zero). Where the “unknown” category was larger than zero, the value refers to women who had no record of referral to the type of follow-up that would be  
618 expected following the recommendations; for them, we assumed that their outcomes would be the same as the outcomes among women who had the correct record of referral. All  
619 other proportions are calculated using values in column “N” as the denominator, as there the “unknown” cells represent e.g. invalid testing outcomes (a normal occurrence in routine  
620 screening which leads to tailored follow-up recommendations).

621 <sup>a</sup> Data from the fourth pilot laboratory which recorded HR-HPV genotyping information using a DNA assay but did not use it for clinical management of HR-HPV positive women.

622 Table 3. Observed distribution of HR-HPV infections and detected CIN2+, by HR-HPV genotype  
 623 and the woman's age.

		Age group			
		24-29	30-49	50-64	Total
	<b>N screened</b>	23,864 (100%)	72,833 (100%)	30,541 (100%)	127,238 (100%)
	<b>HR-HPV genotype at baseline</b>				
<b>HR-HPV infections</b>	<b>HR-HPV+</b>	6709 (28%)	7646 (10%)	1742 (6%)	16,097 (13%)
	<b>HPV 16+</b>	2111 (9%)	1588 (2%)	348 (1%)	4047 (3%)
	<b>Else HPV 18+</b>	509 (2%)	541 (1%)	110 (<1%)	1160 (1%)
	<b>Else other HR-HPV+</b>	4089 (17%)	5517 (8%)	1284 (4%)	10,890 (9%)
<b>Colposcopies</b>	<b>HR-HPV+</b>	4013 (17%)	3890 (5%)	856 (3%)	8759 (7%)
	<b>HPV 16+</b>	1649 (7%)	1125 (2%)	215 (1%)	2989 (2%)
	<b>Else HPV 18+</b>	364 (2%)	325 (<1%)	61 (<1%)	750 (1%)
	<b>Else other HR-HPV+</b>	2000 (8%)	2440 (3%)	580 (2%)	5020 (4%)
<b>CIN2+</b>	<b>HR-HPV+</b>	1579 (7%)	1133 (2%)	147 (<1%)	2859 (2%)
	<b>HPV 16+</b>	899 (4%)	475 (1%)	49 (<1%)	1423 (1%)
	<b>Else HPV 18+</b>	138 (1%)	95 (<1%)	14 (<1%)	247 (<1%)
	<b>Else other HR-HPV+</b>	542 (2%)	563 (1%)	84 (<1%)	1189 (1%)
<b>CIN3+</b>	<b>HR-HPV+</b>	980 (4%)	699 (1%)	84 (<1%)	1763 (1%)
	<b>HPV 16+</b>	613 (3%)	324 (<1%)	31 (<1%)	968 (1%)
	<b>Else HPV 18+</b>	82 (<1%)	62 (<1%)	9 (<1%)	153 (<1%)
	<b>Else other HR-HPV+</b>	285 (1%)	313 (<1%)	44 (<1%)	642 (1%)

624 CIN: cervical intraepithelial neoplasia. HR-HPV: high risk human papillomavirus.

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628 Table 4. Estimated numbers of colposcopies, high-grade CIN, and women not attending the two early recalls following the genotyping and non-  
629 genotyping triage protocols, by time of testing.

Time of testing	Screening test outcome at time of testing	HR-HPV genotyping protocol				Non-genotyping protocol			
		Colposcopies	CIN2+ (PPV)	CIN3+ (PPV)	Not attending early recall	Colposcopies	CIN2+ (PPV)	CIN3+ (PPV)	Not attending early recall
Baseline test	HR-HPV+ and cyt+	5163	2135 (41%)	1367 (26%)		5163	2135 (41%)	1367 (26%)	
Early recall at 12 months	Not attending early recall				1741				1741
	HR-HPV+ and cyt+	1369	479 (35%)	272 (20%)		1369	479 (35%)	272 (20%)	
	HPV 16/18+ and cyt-	1020	133 (13%)	71 (7%)					
Early recall at 24 months	Not attending early recall				637				864
	HPV+	1198	123 (10%)	59 (5%)		1728	221 (13%)	111 (6%)	
Total		8750	2869 (33%)	1769 (20%)	2378	8260	2835 (34%)	1751 (21%)	2626

630 CIN: cervical intraepithelial neoplasia. HR-HPV: high risk human papillomavirus. PPV: positive predictive value.

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633 Table 5. Absolute and relative differences in the numbers of colposcopies, the numbers of detected CIN2+ and CIN3+, and in the numbers of women  
634 not attending early recall between the two triage protocols, by age at screening.

	Absolute numbers per protocol							
	HR-HPV genotyping protocol				Non-genotyping protocol			
Age (years)	Colposcopies (95% CI)	CIN2+ (95% CI)	CIN3+ (95% CI)	Not attending early recall (95% CI)	Colposcopies (95% CI)	CIN2+ (95% CI)	CIN3+ (95% CI)	Not attending early recall (95% CI)
Total <sup>a</sup>	8750 (8572-8924)	2869 (2762-2973)	1769 (1686-1851)	2378 (2283-2475)	8260 (8079-8444)	2835 (2730-2937)	1751 (1668-1832)	2626 (2520-2731)
24-29	4003 (3889-4115)	1588 (1510-1663)	985 (924-1046)	1005 (945-1067)	3780 (3658-3900)	1566 (1489-1640)	973 (913-1033)	1137 (1069-1206)
30-49	3884 (3765-4000)	1135 (1068-1202)	701 (650-754)	1122 (1057-1187)	3665 (3543-3784)	1123 (1057-1189)	694 (644-747)	1221 (1150-1292)
50-64	862 (806-918)	147 (124-172)	84 (66-102)	252 (221-283)	810 (752-867)	146 (123-170)	83 (66-101)	276 (242-311)

	Differences between the protocols							
	Absolute differences (genotyping protocol – non-genotyping protocol)				Relative differences (vs. non-genotyping protocol)			
Age (years)	Colposcopies (95% CI)	CIN2+ (95% CI)	CIN3+ (95% CI)	Not attending early recall (95% CI)	Colposcopies (95% CI)	CIN2+ (95% CI)	CIN3+ (95% CI)	Not attending early recall (95% CI) <sup>b</sup>
Total <sup>a</sup>	+490 (+420 to +562)	+34 (+26 to +43)	+18 (+13 to +24)	-248 (-270 to -226)	+5.9% (+4.4 to +7.7)	+1.2% (+0.6 to +2.0)	+1.0% (+0.5 to +1.8)	-2.3% (-2.5 to -2.1)
24-29	+223 (+174 to +277)	+22 (+15 to +31)	+12 (+7 to +17)	-131 (-150 to -114)	+5.9% (+3.4 to +14.8)	+1.4% (+0.4 to +6.2)	+1.2% (+0.3 to +5.2)	-3.2% (-3.6 to -2.8)
30-49	+219 (+174 to +269)	+12 (+8 to +17)	+7 (+4 to +11)	-99 (-113 to -86)	+6.0% (+4.0 to +8.4)	+1.1% (+0.4 to +2.0)	+1.0% (+0.4 to +2.0)	-1.8% (-2.1 to -1.6)
50-64	+52 (+33 to +74)	+1 (+0 to +3)	+1 (+0 to +2)	-24 (-31 to -18)	+6.4% (+2.8 to +12.3)	+0.9% (+0.1 to +3.0)	+0.7% (+0.0 to +2.7)	-1.8% (-2.3 to -1.4)

636 <sup>a</sup> The totals as reported in Table 4. Sums by age differ slightly due to minor age-specific differences in completeness of follow-up and rounding.

637 <sup>b</sup> Vs. the number of HR-HPV positive cytology negative women at baseline.

638 **FIGURE LEGENDS**

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640 Figure 1. Screening outcomes including colposcopies and detection of CIN2+ outside of the  
641 recommended protocol. Screening was undertaken between May 2013 and December 2014, follow-  
642 up data were retrieved until May 2017. *Panel A.* Women with HPV 16/18 infections at baseline.  
643 *Panel B.* Women with HR-HPV infections other than HPV 16/18 at baseline.

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646 **SUPPLEMENTARY INFORMATION**

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648 Estimation of the additional number of colposcopies in the case of an immediate referral of all HPV  
649 16/18 positive women

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651 Total number of colposcopies in the screened population (with the genotyping protocol): 8750  
652 (Table 4)

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654 Number of HPV 16/18 positive, cytology negative women at baseline: 2914 (Figure 1)

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656 Attendance at colposcopy at baseline: 97.7% (observed for HR-HPV positive cytology positive  
657 women, Table 2)

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659 Estimated needed number of colposcopies for direct referral of HPV 16/18 positive, cytology  
660 negative women: 2846 ( $0.977 \times 2914$ )

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662 Observed number of colposcopies in HPV 16/18 positive, cytology negative women (at any time  
663 during the early recall and including colposcopies outside of the recommended protocol): 1485  
664 (Figure 1)

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666 Difference between the estimated needed and the observed numbers: 1361 (2846-1485)

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668 Relative increase in the number of colposcopies: 16% ( $1361/8750$ )

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673 Estimation of the numbers of detected CIN, colposcopies, and women not attending early recall in the base case analysis

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676 **Table S1.** Observed data. (Note: This is the same as Table 2 in the main text, with the addition of “Code” which simplifies the calculations below.

677 “Unknown” results were excluded from the denominators in cases of referral that deviated from the recommended management protocol.)

Code	Description	Numerator	Denominator	Proportion
	<b>BASELINE</b>			
N	Number of women in the analysis	127,328	NR	NR
P1	HR-HPV+	16,097	127,328	12.6%
P2	Cytology+ if HR-HPV+	5287	16,097	32.8%
P3	Had colposcopy if HR-HPV+/cytology+ after a record of referral	5163	5287	97.7%
Q1	PPV of colposcopy for CIN2+ if HR-HPV+/cytology+	2135	5163	41.4%
Q1b	PPV of colposcopy for CIN3+ if HR-HPV+/cytology+	1367	5163	26.5%
	<b>EARLY RECALL AT 12 MONTHS (HR-HPV+/cytology- at baseline)</b>			
P4	Had testing at 12-month early recall after a record of referral	8964	10,685	83.9%
P5	HR-HPV+	5263	8964	58.7%
P6	Cytology+ if HR-HPV+	1410	5263	26.8%
P7	Had colposcopy if HR-HPV+/cytology+ after a record of referral	1353	1410	96.0%
Q2	PPV of colposcopy for CIN2+ if HR-HPV+/cytology+	473	1353	35.0%
Q2b	PPV of colposcopy for CIN3+ if HR-HPV+/cytology+	269	1353	19.9%
P6a	Cytology- if HR-HPV+	3830	5263	72.8%
P8	HPV 16 or 18+ if HR-HPV+/cytology-	1072	3830	28.0%
P9	Had colposcopy if HPV 16 or 18+/cytology- after a record of referral	789	839	94.0%
Q3	PPV of colposcopy for CIN2+ if HPV 16 or 18+/cytology-	103	789	13.1%
Q3b	PPV of colposcopy for CIN3+ if HPV 16 or 18+/cytology-	55	789	7.0%
	<b>EARLY RECALL AT 24 MONTHS (other HR-HPV +/cytology- at baseline and HR-HPV+/cytology- at 12-month early recall)</b>			
P10	Had testing at 24-month early recall after a record of referral	2091	2710	77.2%
P11	HR-HPV+	1368	2091	65.4%
P12	Had colposcopy after a record of referral	1144	1345	85.1%
Q4	PPV of colposcopy for CIN2+ if HR-HPV+	117	1144	10.2%
Q4b	PPV of colposcopy for CIN3+ if HR-HPV+	56	1144	4.9%
	<b>EARLY RECALL AT 24 MONTHS (HPV 16 or 18+/cytology- at baseline and HR-HPV+/cytology- at 12-month early recall)</b>			
P14	HR-HPV+	73	98	74.5%

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679 **Table S2. Genotyping protocol.** Women with HPV 16/18 infections who remain HR-HPV+/cyt- at 12-month early recall are referred to colposcopy.

680 Women with other HR-HPV infections who remain HR-HPV+/cyt- at 12-month early recall are referred to 24-month early recall.

Time of testing	Screening test outcome at time of testing	Colposcopies	CIN2+	CIN3+	Not attending early recall
Baseline test	HR-HPV+/cytology+	$N \cdot P1 \cdot P2 \cdot P3 = 127328 \cdot (16097/127328) \cdot (5287/16097) \cdot (5163/5287) = 5163$	$N \cdot P1 \cdot P2 \cdot P3 \cdot Q1 = 5163 \cdot (2135/5163) = 2135$	$N \cdot P1 \cdot P2 \cdot P3 \cdot Q1b = 5163 \cdot (1367/5163) = 1367$	
Early recall at 12 months	Not attending				$N \cdot P1 \cdot (1-P2) \cdot (1-P4) = 127328 \cdot (16097/127328) \cdot (1-(5287/16097)) \cdot (1-8964/10685) = 1741$
	HR-HPV+/cytology+	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6 \cdot P7 = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (1410/5263) \cdot (1353/1410) = 1369$	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6 \cdot P7 \cdot Q2 = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (1410/5263) \cdot (1353/1410) \cdot (473/1353) = 479$	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6 \cdot P7 \cdot Q2b = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (1410/5263) \cdot (1353/1410) \cdot (269/1353) = 272$	
	HPV 16 or 18+/cytology-	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6a \cdot P8 \cdot P9 = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (3830/5263) \cdot (1072/3830) \cdot (789/839) = 1020$	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6a \cdot P8 \cdot P9 \cdot Q3 = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (3830/5263) \cdot (1072/3830) \cdot (789/839) \cdot (103/789) = 133$	$N \cdot P1 \cdot (1-P2) \cdot P4 \cdot P5 \cdot P6a \cdot P8 \cdot P9 \cdot Q3b = 16097 \cdot (1-(5287/16097)) \cdot (8964/10685) \cdot (5263/8964) \cdot (3830/5263) \cdot (1072/3830) \cdot (789/839) \cdot (55/789) = 71$	

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682 **Table S2.** Continued.

Time of testing	Screening test outcome at time of testing	Colposcopies	CIN2+	CIN3+	Not attending early recall
Early recall at 24 months	Not attending				$N * P1 * (1 - P2) * P4 * P5 * P6a * (1 - P8) * (1 - P10) = 10810 * (8964 / 10685) * (5263 / 8964) * (3830 / 5263) * (1 - 1072 / 3830) * (1 - 2091 / 2710) = 637$
	HR-HPV+	$N * P1 * (1 - P2) * P4 * P5 * P6a * (1 - P8) * P10 * P11 * P12 = 10810 * (8964 / 10685) * (5263 / 8964) * (3830 / 5263) * (1 - 1072 / 3830) * 2091 / 2710 * (1368 / 2091) * (1144 / 1345) = 1198$	$N * P1 * (1 - P2) * P4 * P5 * P6a * (1 - P8) * P10 * P11 * P12 * Q4 = 10810 * (8964 / 10685) * (5263 / 8964) * (3830 / 5263) * (1 - 1072 / 3830) * 2091 / 2710 * (1368 / 2091) * (1144 / 1345) * (117 / 1144) = 123$	$N * P1 * (1 - P2) * P4 * P5 * P6a * (1 - P8) * P10 * P11 * P12 * Q4b = 10810 * (8964 / 10685) * (5263 / 8964) * (3830 / 5263) * (1 - 1072 / 3830) * 2091 / 2710 * (1368 / 2091) * (1144 / 1345) * (56 / 1144) = 59$	
Total		8750	2870	1769	2378

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688 **Table S3. Non-genotyping protocol.** All women with HR-HPV infections who remain HR-HPV+/cyt- at 12-month early recall are referred to 24-  
689 month early recall (regardless of genotype).

Time of testing	Screening test outcome at time of testing	Colposcopies	CIN2+	CIN3+	Not attending early recall
Baseline test	HR-HPV+/cytology+	$N * P1 * P2 * P3 = 127328 * (16097 / 127328) * (5287 / 16097) * (5163 / 5287) = 5163$	$N * P1 * P2 * P3 * Q1 = 5163 * (2135 / 5163) = 2135$	$N * P1 * P2 * P3 * Q1b = 5163 * (1367 / 5163) = 1367$	
Early recall at 12 months	Not attending				$N * P1 * (1 - P2) * (1 - P4) = 127328 * (16097 / 127328) * (1 - (5287 / 16097)) * (1 - 8964 / 10685) = 1741$
	HR-HPV+/cytology+	$N * P1 * (1 - P2) * P4 * P5 * P6 * P7 = 16097 * (1 - (5287 / 16097)) * (8964 / 10685) * (5263 / 8964) * (1410 / 5263) * (1353 / 1410) = 1369$	$N * P1 * (1 - P2) * P4 * P5 * P6 * P7 * Q2 = 16097 * (1 - (5287 / 16097)) * (8964 / 10685) * (5263 / 8964) * (1410 / 5263) * (1353 / 1410) * (473 / 1353) = 479$	$N * P1 * (1 - P2) * P4 * P5 * P6 * P7 * Q2b = 16097 * (1 - (5287 / 16097)) * (8964 / 10685) * (5263 / 8964) * (1410 / 5263) * (1353 / 1410) * (269 / 1353) = 272$	
Early recall at 24 months	Not attending				$N * P1 * (1 - P2) * P4 * P5 * P6a * (1 - P10) = 10810 * (8964 / 10685) * (5263 / 8964) * (3830 / 5263) * (1 - (2091 / 2710)) = 885$

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692 **Table S3.** Continued.

Time of testing	Screening test outcome at time of testing	Colposcopies	CIN2+	CIN3+	Not attending early recall
	HR-HPV+	$N \times P1 \times (1 - P2) \times P4 \times P5 \times P6a \times P10 \times ((1 - P8) \times P11 + P8 \times P14) \times P12 = 10810 \times (8964/10685) \times (5263/8964) \times (3830/5263) \times (2091/2710) \times ((1 - (1072/3830)) \times (1368/2091) + (1072/3830) \times (73/98)) \times 1144/1345 = 1728$ <i>HR-HPV other: 1198</i> <i>HPV 16/18: 530</i>	$N \times P1 \times (1 - P2) \times P4 \times P5 \times P6a \times (1 - P8) \times P10 \times P11 \times P12 \times Q4 + N \times P1 \times (1 - P2) \times P4 \times P5 \times P6a \times P8 \times P10 \times Q3 \times (1/P9) \times P12 = 10810 \times (8964/10685) \times (5263/8964) \times (3830/5263) \times (1 - 1072/3830) \times 2091/2710 \times (1368/2091) \times (1144/1345) \times (117/1144) + 10810 \times (8964/10685) \times (5263/8964) \times (3830/5263) \times (1072/3830) \times (2091/2710) \times (103/789) \times (839/789) \times (1144/1345) = 221$ <i>HR-HPV other:</i> <i>1198 \times (117/1144) = 123</i> <i>HPV 16/18:</i> <i>1085 \times (103/789) \times (839/789) \times (1144/1345) \times (2091/2710) = 99</i>	$N \times P1 \times (1 - P2) \times P4 \times P5 \times P6a \times (1 - P8) \times P10 \times P11 \times P12 \times Q4b + N \times P1 \times (1 - P2) \times P4 \times P5 \times P6a \times P8 \times P10 \times Q3b \times (1/P9) \times P12 = 10810 \times (8964/10685) \times (5263/8964) \times (3830/5263) \times (1 - 1072/3830) \times 2091/2710 \times (1368/2091) \times (1144/1345) \times (56/1144) + 10810 \times (8964/10685) \times (5263/8964) \times (3830/5263) \times (1072/3830) \times (2091/2710) \times (55/789) \times (839/789) \times (1144/1345) = 111$ <i>HR-HPV other:</i> <i>1198 \times (56/1144) = 59</i> <i>HPV 16/18:</i> <i>1084 \times (55/789) \times (839/789) \times (1144/1345) \times (2091/2710) = 53</i>	
Total		8260	2835	1750	2626

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